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EXAMINER	
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1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/585,620	LIU ET AL.	
	Examiner	Art Unit	
	GANAPATHIRAMA RAGHU	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 July 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *18-36* is/are pending in the application.

4a) Of the above claim(s) *30-35* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *18,19,23-29 and 36* is/are rejected.

7)☒ Claim(s) *18-29 and 36* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *07/08/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

Election/Restriction

Applicant's election with traverse of Group I, claims 18-29 and 36 with respect to SEQ ID NO: 2 for prosecution in the reply filed on dated 07/22/2008 is acknowledged Applicants have elected said group with the proviso that applicants reserve the right to file DIV applications to the non-elected subject matter.

The traversal is on the ground(s) that "in regard to the species election, the Examiner states that the sequences have different structure and function. Applicants respectfully submit that the sequences clearly have highly similar structures and function as both are highly related sequences derived from the same organism". This is not found persuasive because: 1) Examiner in his reasons for restriction in the letter dated 04/03/08 had clearly stated that "this is a restriction requirement to sequence and NOT a species election and it has been determined that 1(ONE) sequence constitutes a reasonable number for examination purposes under the present conditions. At present the huge number of submissions of claims directed to various sequences, such as nucleic acids or polypeptides, is so large that the election of sequence of this type is now deemed to be practically appropriate so as to not overwhelm the examination and search processes for such claims. Examination will be restricted to only the elected group and the elected amino acid /nucleotide sequence; and 2) Furthermore, searching structurally distinct polypeptides of Group I and their encoding polynucleotides are not coextensive and involves search of different databases and non-patent literature, as prior to the concomitant isolation and expression of the sequence of interest there may be scientific journal articles devoted solely to the polypeptide which would not have

described related polypeptides or the encoding polynucleotide and moreover the polypeptides may have been isolated by biochemical means from natural source or generated by peptide synthesis methods as opposed to the expression of polypeptide through recombinant methods. In addition, Group I polypeptides and encoding polynucleotides encompasses molecules which are claimed in terms of mutants and variants of SEQ ID NO: 2 and encoding polynucleotide sequence of SEQ ID NO: 1 having at least 70% sequence homology to the polypeptide and the encoding polynucleotide, hence the breadth and the scope of the claims are very broad that involves search of different sequence databases and analysis of results.

Therefore, for the above cited reasons searching of additional sequences that are patentably distinct in Group I is a serious search burden and contrary to applicant's argument, the requirement is still deemed proper and is therefore made FINAL.

Claims 18-36 are pending in this application, claims 18-29 and 36 with respect to SEQ ID NO: 2 encoded by the polynucleotide sequence of SEQ ID NO: 1 are now under consideration for examination. Claims 30-35 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected inventions, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 07/22/2008.

Priority

Acknowledgment is made of applicant's claim for foreign priority under 35 U.S.C. 119(a)-(d). This application is a 371 of PCT/DK04/00896 filed on 12/22/2004, which claims the priority of Demark Application No.: PA 2004-00021 filed on 01/08/2004.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 07/08/2006 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the examiner is considering the IDS statement.

Objections to Abstract

The Abstract of the disclosure is objected to because, Abstract should be on a separate sheet of paper. Correction is required. See MPEP § 608.01(b).

Claim Objections

Claims 18-29 and 36 are objected to, due to the following informality: Claims 18-29 and 36 recite non-elected subject-matter i.e., SEQ ID NO: 18 and 19. Examiner also notes that claim 18 recites *Valsaria rubricosa* CBS 848.96 strain that comprises the polynucleotide of SEQ ID NO: 18 (page 4 of specification) and therefore deemed as non-elected subject matter. Appropriate correction is required.

Claims 26-28 are objected to, due to the following informality: Claims 26-28 recite "a vector comprising the polynucleotide of claim 18". Examiner notes that claim 18 is directed to an isolated polypeptide and not to a polynucleotide. Appropriate correction is required.

Claim Rejections: 35 USC § 112-First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Enablement

Claims 18, 19, 23-29 and 36 are rejected under 35 U.S.C. 112, first paragraph, because the specification while being enabling for an isolated polypeptide having amylase activity, said polypeptide having 90% sequence identity to the amino acid

residues 1-439 or 1-566 of SEQ ID NO: 2 and encoded by a polynucleotide sequence comprising the nucleotides 146-1462 of SEQ ID NO: 1, vector comprising said polynucleotide, isolated host cell, method of making said polypeptide and compositions comprising said polypeptide, does not reasonably provide enablement for any polypeptide having amylase activity, said polypeptide having i) a catalytic core encoded by a DNA sequence (as in claim 18 part (a)) or a sequence shown in positions 1-439 or position 1-566 of SEQ ID NO: 2 (as in claim 18 part (b)) or a polypeptide sequence having 70%-80% sequence identity to the amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 and encoded by a polynucleotide sequence which hybridizes under recited stringent conditions and wash conditions to the full complement of a polynucleotide sequence comprising the nucleotides 146-1462 of SEQ ID NO: 1 twice for 30 minutes in 2 x SSC, 0.5% SDS at a temperature of 60^0C (as in claim 18 part (c) and claim 19); ii) twice for 30 minutes in 2 x SSC, 0.5% SDS at a temperature of 65^0C (as in claim 23); iii) twice for 30 minutes in 2 x SSC, 0.5% SDS at a temperature of 70^0C (as in claim 24); iv) said polypeptide further comprising a carbohydrate-binding domain (as in claim 25) and v) vector comprising said polynucleotide, host cell, method of making said polypeptide and compositions comprising said polypeptide (as in claims 26-29 and 36). The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with the claims.

Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir.

1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

The breadth of the claims: Claims 18, 19, 23-29 and 36 are so broad as to encompass any polypeptide having amylase activity, said polypeptide having i) a catalytic core encoded by a DNA sequence (as in claim 18 part (a)) or a sequence shown in positions 1-439 or position 1-566 of SEQ ID NO: 2 (as in claim 18 part (b)) or a polypeptide sequence having 70%-80% sequence identity to the amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 and encoded by a polynucleotide sequence which hybridizes under recited stringent conditions and wash conditions to the full complement of a polynucleotide sequence comprising the nucleotides 146-1462 of SEQ ID NO: 1 twice for 30 minutes in 2 x SSC, 0.5% SDS at a temperature of 60^0C (as in claim 18 part (c) and claim 19); ii) twice for 30 minutes in 2 x SSC, 0.5% SDS at a temperature of 65^0C (as in claim 23); iii) twice for 30 minutes in 2 x SSC, 0.5% SDS at a temperature of 70^0C (as in claim 24); iv) said polypeptide further comprising a carbohydrate-binding domain (as in claim 25) and v) vector comprising said polynucleotide, host cell, method of making said polypeptide and compositions comprising said polypeptide (as in claims 26-29 and 36). This rejection is made because Office contends: i) It should be noted that claim 18 part (a) and (b) is being directed to "a DNA sequence" and "a sequence" and as such, the term "a sequence" in broadest reasonable interpretation encompasses

not only amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 but any fragment within SEQ ID NO: 2 due to the recitation of "a sequence" (given that the claim language recites "a sequence" and "a DNA sequence", it is deemed to encompass and read on "any two/dipeptide sequences of SEQ ID NO: 2 and encoded by "any six corresponding encoding nucleotides"); and ii) that hybridization conditions recited in part (d) of claim 18 and claims 23 and 24 are considered as low-medium stringency conditions of hybridization, which would also allow unrelated (low homology sequences) nucleic acid sequence(s) to hybridize to nucleic acid sequences as claimed in claims 18, 23, 24 and 25. The specification on page 4, lines 20-30 states the recited hybridization conditions but does define the type of stringency encompassed by the recited conditions. The hybridization conditions are of low-medium stringency (high salt wash: **2 x SSC**) and this will allow many heterogeneous population of nucleic acid molecules, i. e., heteroduplexes including many mutants, variants and recombinants of said polynucleotides to hybridize to target molecule of 146-1462 of SEQ ID NO: 1 at said stringent conditions (see Current Protocols in Molecular Biology, 1993, pages 2.10.1-2.10.16) and said polynucleotides may not necessarily encode a functionally active amylase. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and or use the invention commensurate in scope with the claim.

The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to extremely large number of polypeptides and encoding polynucleotides broadly encompassed by the claims. Since the amino acid sequence of

a protein encoded by a polynucleotide determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires knowledge and guidance with regard to which amino acids in the protein's sequence and the respective codons in its polynucleotide, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the encoded proteins' structure relates to its function. It may be emphasized that it was very well established in the art (Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1982), see in particular, pages 387-389) at the time the claimed invention was made that in order to prevent hybridization of unrelated nucleic acid sequence(s) to a target sequence, hybridization and subsequent washing conditions must be highly stringent. For example, hybridization under conditions of 0.1-1.0 x SSC, 50% formamide and 50^0C for 24 hours, followed by 2 washes in 0.1% SDS, 0.1 x SSC at 65^0C for 25-30 minutes each is considered highly stringent condition that would not allow hybridization of unrelated nucleic acid sequences to the target sequence.

The amount of direction or guidance presented and the existence of working examples: The specification is limited to teaching the use of an isolated polypeptide having amylase activity, said polypeptide comprising the amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 and encoded by a polynucleotide sequence comprising the nucleotides 146-1462 of SEQ ID NO: 1, vector comprising said polynucleotide, isolated host cell, method of making said polypeptide and compositions

comprising said polypeptide, but provides no guidance with regard to the making of other variants and mutants with amylase activity or with regard to other uses. In view of the great breadth of the claims, amount of experimentation required to make the claimed polypeptides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure (for example, see Whisstock et al., Prediction of protein function from protein sequence and structure. Q Rev Biophys. 2003, Aug. 36 (3): 307-340. Review), the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to make and use the full scope of the polypeptides and encoding polynucleotides encompassed by these claims.

The state of prior at, the relative skill of those in the art, and the predictability or unpredictability of the art: While enzyme isolation techniques, recombinant and mutagenesis techniques are known, and it is not routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claim, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable (e.g., see Whisstock et al., Q Rev Biophys. 2003 Aug; 36(3): 307-340). In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions or deletions.

Claims 18, 19, 23-29 and 36 as written are directed to random variant and

mutant polypeptides having amylase activity, said polypeptide encoded by random mutants and variants of a polynucleotide comprising nucleotides 146-1462 of SEQ ID NO: 1, i.e., any polypeptide having amylase activity, said polypeptide having i) a catalytic core encoded by a DNA sequence (as in claim 18 part (a)) or a sequence shown in positions 1-439 or position 1-566 of SEQ ID NO: 2 (as in claim 18 part (b)) or a polypeptide sequence having 70%-80% sequence identity to the amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 and encoded by a polynucleotide sequence which hybridizes under recited stringent conditions and wash conditions to the full complement of a polynucleotide sequence comprising the nucleotides 146-1462 of SEQ ID NO: 1 twice for 30 minutes in 2 x SSC, 0.5% SDS at a temperature of 60^0C (as in claim 18 part (c) and claim 19); ii) twice for 30 minutes in 2 x SSC, 0.5% SDS at a temperature of 65^0C (as in claim 23); iii) twice for 30 minutes in 2 x SSC, 0.5% SDS at a temperature of 70^0C (as in claim 24); iv) said polypeptide further comprising a carbohydrate-binding domain (as in claim 25) and v) vector comprising said polynucleotide, host cell, method of making said polypeptide and compositions comprising said polypeptide (as in claims 26-29 and 36). However, any polypeptide having amylase activity, said polypeptide having 70%-80% sequence identity to the amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 and encoded by a polynucleotide sequence which hybridizes under recited stringent conditions and wash conditions to the full complement of a polynucleotide sequence comprising the nucleotides 146-1462 of SEQ ID NO: 1: i) twice for 30 minutes in **2 x SSC**, 0.5% SDS at a temperature of 60^0C (as in claim 18); ii) twice for 30 minutes in **2 x SSC**, 0.5% SDS at a temperature of 65^0C (as in claim 23); iii) twice for 30 minutes

in **2 x SSC**, 0.5% SDS at a temperature of 70^{0}C (as in claim 24), would clearly constitute **undue** experimentation.

It is also noted that the art teaches several examples of how even small changes in structure can lead to changes in function. For example, Witkowski et al. (Biochemistry, 1999, Vol. 38: 11643-116150) teaches that one conservative amino acid substitution transforms a β-ketoacyl synthase into a malonyl decarboxylase and completely eliminates β-ketoacyl synthase activity. Seffernick et al. (J. Bacteriol., 2001, Vol. 183 (8): 2405-2410) teaches that two naturally occurring Pseudomonas enzymes having 98% amino acid sequence identity catalyze two different reactions: deamination and dehalogenation, therefore having different function.

The quantity of experimentation required to practice the claimed invention based on the teachings of the specification: Factors influencing hybrid stability are well known in the art (Brown T., Current protocols in Molecular Biology, 1993: 2.10.1-2.10.16, publishers John Wiley & Sons, Inc. New York, USA). Hybrid stability is expressed as the melting temperature T_m, which is the temperature at which the probe dissociates from the target DNA. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl (1984):

T_m = 81.5^{0}C + 16.6(log*M*) + 0.41(%GC) - 0.61(%form) - 500/L (page 2.10.8 of Brown T., Current protocols in Molecular Biology, 1993). Where *M* is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, %form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. Also note that the instant hybridization and wash

conditions recited in the instant claims do not require formamide and therefore that factor is not included in the calculation for T_m. Therefore, for purposes of calculation the equation is as follows: $T_m = 81.5^0C + 16.6(\log M) + 0.41(\%GC) - 500/L$. The molarity of monovalent cations *M* in 2 x SSC (final wash conditions defined in the instant claims 18, 23 and 24) is given by the formula 3.9 M X 2/20 = 0.39 M, wherein the molarity of 20 x SSC = 3.9 M (see Table 2.10.3 in page 2.10.11 of Brown T., Current protocols in Molecular Biology, 1993). The percentage of GC content of SEQ ID NO: 1 comprising the nucleotides 146-1462 (total number of nucleotides 1316) was determined to be 63%. Therefore the T_m for SEQ ID NO: 1 comprising nucleotides 146-1462 as determined by the formula is $81.5^0C + 16.6(\log 0.39) + 0.41(63) - 500/1316 = 81.5^0C - 6.7 + 25.83 - 0.37 = 100.26^0C$ or **~ 100.0^{0}C**. Brown T., (Current protocols in Molecular Biology, 1993) further gives guidance regarding designing washing for heteroduplex hybridization and the degree of mismatching that will be tolerated by a "moderate- or low-stringency" wash. "The best way to approach this is to first establish the lowest temperature at which only homologous hybridization occurs with a particular SSC concentration and then assume that 1% mismatching results in a decrease of 1^0C in the T_m and reduce the temperature of the final wash accordingly, for example if sequences with ≥90% similarity with the probe are being sought, decrease the final wash by 10^0C" (column 1, page 2.10.11). Applying the guidance provided by Brown T., (Current protocols in Molecular Biology, 1993) the washing conditions cited in the instant claims i. e., **2 x SSC** at 60^0C will yield heteroduplexes that have 60% sequence identity to SEQ ID NO: 1 ($100^0C - 60^0C = 40^0C$ lower than T_m and that translates to 100% - 40% =

60% sequence identity, for every 1% mismatching results in a decrease of 1^0C in the T_m; similarly **2 x SSC** at 65^0C will yield heteroduplexes that have 65% sequence identity to SEQ ID NO: 1 and for **2 x SSC** at 70^0C will yield heteroduplexes that have 70% sequence identity to SEQ ID NO: 1).

While methods to produce variants of a known sequence, such as site-specific mutagenesis, random mutagenesis, etc., are well known to the skilled artisan, producing variants as claimed requires that one of ordinary skill in the art know or be provided with guidance for the selection of which, of the infinite number of variants, have the activity. As argued above, claims 18, 19, 23-29 and 36 as written are directed to random variants and mutant polypeptides of SEQ ID NO: 2 having amylase activity and encoded by random mutants and variants of a polynucleotide comprising a nucleotide sequence of SEQ ID NO: 1. The guidance provided by the applicants is limited to the specific residues of SEQ ID NO: 1 and not to any random changes in the encoding polynucleotide of SEQ ID NO: 1, said polynucleotide having about 60%-70% sequence identity to SEQ ID NO: 1 (as per the determined % identity by the above calculation method) or said polypeptide having 70%-80% sequence identity to the amino acid residues 1-439 or 1-566 of SEQ ID NO: 2. For the rejected claims, this would clearly constitute **undue** experimentation.

Additionally, Guo et al., (PNAS, 2004, Vol. 101 (25): 9205-9210) teach that the percentage of random single-substitution mutations, which inactivate a protein, using a protein 3-methyladenine DNA glycosylase as a model, is 34% and that this number is consistent with other studies in other proteins (p 9206, paragraph 4). Guo et al., (*supra*)

further show that the percentage of active mutants for multiple mutations/changes appears to be exponentially related to this by the simple formula $(0.66)^{x}$ X 100% where **x** is the number of mutations introduced (Table 1). Applying this estimate to the protein recited in the instant application, any polynucleotide sequence which is at least 60%-70% homologous to the polynucleotide of SEQ ID NO: 1 and encoding a polypeptide having amylase activity as claimed in claims 18, 19, 23-29 and 36 allows up to 566 mutations/changes within the 1316 nucleotides of the encoding polynucleotide sequence of SEQ ID NO: 1 and, thus, only $(0.66)^{526}$ X 100% equivalent to **1.2×10^{-93}%** of random mutants and encoded by a polynucleotide having 60% homology to the polynucleotide of SEQ ID NO: 1 would be active. Further, if one assumes that the guidance provided by the teaching of the catalytic sites, comparison of known active homologous sequences and available modeling techniques (as taught by the art for amylases) would allow a skilled artisan to avoid mutating residues with a high probability of inactivating the amylase activity and thus the estimate of percentage of single substitution random mutations which inactivate the instant encoded polypeptide could be improved to only 20%, the above formula then becomes $(0.8)^{x}$ X 100%. Applying this estimate to the protein recited in the instant application, $(0.8)^{526}$ X 100% or **1.6×10^{-49} %** of **guided** mutants having 60% sequence homology to the encoding polynucleotide of SEQ ID NO: 1 would be active. While these calculations are only estimates of the actual situation, they are presented to provide a basis for understanding the examiner's decision on which claim scope would require only routine experimentation and which claim scope would reach a level which is undue. Examiner

has tabulated the estimated probability of finding an active mutant/variant among extremely large number of inactive mutants is shown below in the following Tables: 1-3:

Table 1: SEQ ID NO: 1 comprising 1316 nucleotides

% Homology	Number of nucleotide changes	Expected active mutants $(0.66)^{X}$ X 100%	Expected active-guided mutants $(0.8)^{X}$ X 100%
60%	526	1.2×10^{-93}%	1.6×10^{-49}%
65%	461	6.4×10^{-82}%	2.1×10^{-43}%
70%	395	5.2×10^{-70}%	5.2×10^{-37}%

Table 2: SEQ ID NO: 2 polypeptide comprising 566 amino acids

% Homology	Number of amino acid changes	Expected active mutants $(0.66)^{X}$ X 100%	Expected active-guided mutants $(0.8)^{X}$ X 100%
80%	114	2.6×10^{-19}%	8.9×10^{-10}%
90%	57	5.1×10^{-9}%	**2.9×10^{-4}%**
98%	11	1.03%	**8.58%**

Table 3: SEQ ID NO: 2 polypeptide comprising 439 amino acids

% Homology	Number of amino acid changes	Expected active mutants $(0.66)^{X}$ X 100%	Expected active-guided mutants $(0.8)^{X}$ X 100%

80%	88	1.3×10^{-14}%	2.9×10^{-7}%
90%	44	1.1×10^{-6}%	**0.005%**
98%	9	2.37%	**13.4%**

The guidance in the instant case and current techniques in the art (i.e., high throughput mutagenesis and screening techniques) would allow for finding a reasonable number of active mutants within hundred thousand inactive mutants as in 90%-98% sequence identity to the polypeptide sequence of SEQ ID NO: 2 and the encoding polynucleotide sequence of SEQ ID NO: 1, estimated guided mutants that would be active ranges from **2.9×10^{-4}%-13.4%**. But finding a few mutants within several trillions or more, as in the claims to 60%-70% sequence identity to the encoding polynucleotide sequence of SEQ ID NO: 1 or 70%-80% sequence identity to the amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 would not be possible. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed (guided mutants). Such guidance has **not** been provided in the instant specification.

Therefore, the specification does not support the broad scope of the claims which encompass any polypeptide having amylase activity, said polypeptide having 70%-80% sequence identity to the amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 and encoded by a polynucleotide sequence which hybridizes under recited stringent conditions and wash conditions to the full complement of a polynucleotide sequence

comprising the nucleotides 146-1462 of SEQ ID NO: 1: i) twice for 30 minutes in **2 x SSC**, 0.5% SDS at a temperature of 60^0C; ii) twice for 30 minutes in **2 x SSC**, 0.5% SDS at a temperature of 65^0C; iii) twice for 30 minutes in **2 x SSC**, 0.5% SDS at a temperature of 70^0C, vector comprising said polynucleotide, host cell, method of making said polypeptide and compositions comprising said polypeptide, as claimed in claims 18, 19, 23-29 and 36, because the specification does not establish: (A) a rational and predictable scheme for modifying specific nucleotides in the polynucleotide sequence of SEQ ID NO: 1 and encoding a polypeptide comprising an amino acid sequence of SEQ ID NO: 2 and said polypeptide having alpha amylase activity (B) regions of the protein/polynucleotide structure which may be modified without affecting the desired biological activity of the encoded polypeptide; (C) the general tolerance of the polypeptide and the encoding polynucleotide to modification and extent of such tolerance; (D) the tertiary structure of the molecule and folding patterns that are essential for the desired biological activity and tolerance to modifications; and (E) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claim broadly including polypeptides and encoding polynucleotides with an enormous number of modifications. The scope of the claim must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1975)). Without sufficient guidance, determination of polynucleotides and

encoded polypeptides having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim 18 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Claim 18 recites a plasmid present in *E.coli* DSM 16113 or in *Valsaria rubricosa* CBS 848.96. However examiner notes that *Valsaria rubricosa* CBS 848.96 harbors the plasmid comprising the non-elected sequence SEQ ID NO: 18 (page 4 of specification).

It is apparent that the plasmid present in *E.coli* DSM 16113 comprising the polynucleotide sequence of SEQ ID NO: 1 is required to practice the claimed invention. As such the biological material must be readily available or obtainable by a repeatable method set forth in the specification, or otherwise readily available to the public. If it is not so obtainable or available, the requirements of 35 USC112, first paragraph, may be satisfied by a deposit of a plasmid present in *E.coli* DSM 16113. The specification does not disclose a repeatable method to obtain these plasmids contained in *E.coli* DSM 16113. It is noted that applicants have deposited the plasmid present in *E.coli* DSM 16113 on 12/16/2003 under the terms of the Budapest Treaty with the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkuturen GmbH, Mascheroder Weg 1B, D-

38124 Braunschweig, Germany, with following deposit Accession Number: DSMZ 16113 (pages 2 and 14 of specification), but there is no indication in the specification as to the public availability. Since the deposit was made under the terms of Budapest Treaty, then a statement, affidavit or declaration by Applicants, or a statement by an attorney of record over his/her signature and registration number, or someone empowered to make such a statement, stating that the invention will be irrevocably and without restriction released to the public upon the issuance of a patent, would satisfy the deposit requirement made herein. In order to certify that the deposit meets the criteria set forth in 37 CFR 1.801-1.809 and MPEP 2402-2411.05, applicant may provide assurance of compliance by statement, affidavit or declaration, or by someone empowered to make same, or by a statement by an attorney of record over his /her signature and registration number showing that:

(a) during the pendency of the application, access to the invention will be afforded to the Commissioner upon request;

(b) all restrictions upon availability to the public will be irrevocably removed upon granting the patent;

(c) the deposit will be maintained in public depository for a period of 30 years, or 5 years after the last request or for the enforceable life of the patent, whichever is longer;

(d) a test of the viability of the biological material at the time of deposit (se 37 CFR 1.807); and the deposit will be replaced if it should ever become inviable.

Claim 27 (directed to a transformed host cell comprising the recombinant polypeptide of SEQ ID NO: 2 encoded by a polynucleotide of SEQ ID NO: 1) is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement when given the broadest reasonable interpretation, because, while claim 27 is enabling for an isolated transformed host cell transformed with synthetic nucleic acid of SEQ ID NO: 1 acid and encoding the recombinant polypeptide of SEQ ID NO: 2 as claimed, does not reasonably provide enablement for transgenic multi-cellular organisms or host cells within a multi-cellular organism that have been transformed with the synthetic nucleic acid. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with the claim.

Claim 27 is so broad as to encompass transgenic multi-cellular organisms and host cells comprising the recombinant polypeptide of SEQ ID NO: 2 and transformed with specific nucleic acids comprising the encoding polynucleotide of SEQ ID NO: 1, including cells in *vitro* culture as well as within any multi-cellular organism. The scope of the claim is not commensurate with the enablement provided by the disclosure with regard to extremely large number of transformed organisms broadly encompassed by the claims. While methods for transforming cells *in vitro* are well known in the art, methods for successfully transforming cells within complex multi-cellular organisms are not routine and are highly unpredictable. Furthermore, methods for producing a successfully transformed cell within the multi-cellular organism are unlikely to be applicable to transformation of other types of multi-cellular organism as multi-cellular

organisms vary widely. However, in this case the disclosure is limited to only host cells *in vitro*. Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including the use of host cells within a multi-cellular organism for the production of polypeptide. The scope of claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA)). Without sufficient guidance, expression of genes in a particular host cell and having the desired biological characteristics is unpredictable, the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F. 2d 731, 8 USPQ 2nd 1400 (Fed. Cir., 1988). It is suggested that the applicants limit the claim to " An isolated transformed host cell ...".

Written Description

Claims 18, 25-29 and 36 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 18, 25-29 and 36 are directed to encompass any polypeptide having amylase activity, said polypeptide having i) a catalytic core encoded by a DNA sequence (as in claim 18 part (a)) or a sequence shown in positions 1-439 or position 1-566 of SEQ ID NO: 2 (as in claim 18 part (b)); ii) said polypeptide further comprising a carbohydrate-binding domain (as in claim 25) and iii) vector comprising said

polynucleotide, host cell, method of making said polypeptide and compositions comprising said polypeptide (as in claims 26-29 and 36).

In *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1938, the Court of Appeals for the Federal Circuit has held that "A written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials". As indicated in MPEP § 2163, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in possession of the claimed genus. In addition, MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

In the instant case, there is no structure-function correlationship recited with regard to the members of the genus of polypeptides and encoding polynucleotides and the specification fails to provide any polypeptide structure having amylase activity and encoding polynucleotide sequence i.e., any polypeptide having amylase activity, said polypeptide having i) a catalytic core encoded by a DNA sequence (as in claim 18 part (a)) or a sequence shown in positions 1-439 or position 1-566 of SEQ ID NO: 2 (as in claim 18 part (b)); ii) said polypeptide further comprising a carbohydrate-binding domain (as in claim 25) and iii) vector comprising said polynucleotide, host cell, method of making said polypeptide and compositions comprising said polypeptide (as in claims 26-29 and 36), because Office contends: i) It should be noted that claim 18 part (a) and (b) is being directed to "a DNA sequence" and "a sequence" and as such, the term "a sequence" in broadest reasonable interpretation encompasses not only amino acid

residues 1-439 or 1-566 of SEQ ID NO: 2 but any fragment within SEQ ID NO: 2 due to the recitation of "a sequence" (given that the claim language recites "a sequence" and "a DNA sequence", it is deemed to encompass and read on "any two/dipeptide sequences of SEQ ID NO: 2 and encoded by "any six corresponding encoding nucleotides").

Therefore, examiner takes the position that due to the paucity of information regarding structure-function correlation, the specification lacks identifying characteristics of all of the sequences within the claimed genus i.e., any polypeptide having amylase activity, said polypeptide having i) a catalytic core encoded by a DNA sequence (as in claim 18 part (a)) or a sequence shown in positions 1-439 or position 1-566 of SEQ ID NO: 2 (as in claim 18 part (b)); ii) said polypeptide further comprising a carbohydrate-binding domain (as in claim 25) and iii) vector comprising said polynucleotide, host cell, method of making said polypeptide and compositions comprising said polypeptide (as in claims 26-29 and 36).

No information, beyond the characterization of an isolated polypeptide having amylase activity, said polypeptide comprising the amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 and encoded by a polynucleotide sequence comprising the nucleotides 146-1462 of SEQ ID NO: 1, vector comprising said polynucleotide, isolated host cell, method of making said polypeptide and compositions comprising said polypeptide has been provided by the applicants, which would indicate that they had possession of the claimed genus of polynucleotides and encoded polypeptides i. e., within the claimed genus.

The genus of polypeptides and encoding polynucleotides required in the claimed invention is an extremely large structurally and functionally variable genus. While the argument can be made that the recited genus of polypeptides and encoding polynucleotides is adequately described by the disclosure of the structure of an isolated polynucleotide of SEQ ID NO: 1 and encoding a protein comprising the amino acid sequence of SEQ ID NO: 2 having amylase activity, since one could use structural homology to isolate those polynucleotides and encoding polypeptides recited in the claims. As taught by the art and cited in the enablement rejection, even highly structurally homologous polynucleotides and encoded polypeptides do not necessarily share the same function i.e., conservation of structure is not necessarily a surrogate for conservation of function. For example, Witkowski et al., (Biochemistry 38:11643-11650, 1999), teaches that one conservative amino acid substitution transforms a β-ketoacyl synthase into a malonyl decarboxylase and completely eliminates β-ketoacyl synthase activity. Seffernick et al., (J. Bacteriol. 183(8): 2405-2410, 2001), teaches that two naturally occurring *Pseudomonas* enzymes having 98% amino acid sequence identity catalyze two different reactions: deamination and dehalogenation, therefore having different function. Broun et al., (Science 282:1315-1317, 1998), teaches that as few as four amino acid substitutions can convert an oleate 12-desaturase into a hydrolase and as few as six amino acid substitutions can transform a hydrolase to a desaturase. Therefore, the claimed genera of polypeptides and encoding polynucleotides include widely variable structure and associated functions, since minor changes in structure

may result in changes affecting function and no additional information correlating structure has been provided.

Due to the fact that the specification only discloses an isolated polypeptide having amylase activity, said polypeptide comprising the amino acid sequence of SEQ ID NO: 2 and encoded by the polynucleotide sequence of SEQ ID NO: 1, and the lack of description of any additional species/variants/mutants/recombinants by any relevant, identifying characteristics or properties or structure-function correlationship for the recited amylase activity, one of skill in the art would not recognize from the disclosure that applicant was in possession of the claimed invention at the time the instant application was filed. Applicants are referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claim Rejections 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 18, 26-29 and 36 are rejected under 35 U.S.C. 102(b) as being anticipated by Michelsen et al., (WO 96/01323 in IDS) when given the broadest reasonable interpretation. Claims 18, 26-29 and 36 are directed to any polypeptide having amylase activity, said polypeptide having i) a catalytic core encoded by a DNA sequence (as in claim 18 part (a)) or a sequence shown in positions 1-439 or position 1-

566 of SEQ ID NO: 2 (as in claim 18 part (b)); and ii) vector comprising said polynucleotide, host cell, method of making said polypeptide and compositions comprising said polypeptide (as in claims 26-29 and 36). As indicated previously, claim 18 part (a) and (b) is being directed to "a DNA sequence" and "a sequence" and as such, the term "a sequence" in broadest reasonable interpretation encompasses not only amino acid residues 1-439 or 1-566 of SEQ ID NO: 2 but any fragment within SEQ ID NO: 2 due to the recitation of "a sequence" (given that the claim language recites "a sequence" and "a DNA sequence", it is deemed to encompass and read on "any two/dipeptide sequences of SEQ ID NO: 2 and encoded by "any six corresponding encoding nucleotides").

Michelsen et al., (*supra)* disclose the isolation of a polypeptide and encoding polynucleotide having amylase activity, including vector, host cell, method of making said polypeptide and use of the said polypeptide in dough compositions and baking (Abstract section, entire document, especially pages 47-50), said reference polypeptide comprises fragments of SEQ ID NO: 2 of the instant application, for example, the following amino acid residues of the reference polypeptide (SEQ ID NO: 2); 10-11 and 22-23 correspond to "Pro Val" and "Ala Ala" which is a dipeptide found in SEQ ID NO: 2 of the instant application at positions 81-82 and 224-225 respectively. Therefore the reference of Michelsen et al., is deemed to anticipate the claims 18, 26-29 and 36 as written.

Allowable Subject Matter/Conclusion

None of the claims are allowable.

Final Comments

To insure that each document is properly filed in the electronic file wrapper, it is requested that each of amendments to the specification, amendments to the claims, Applicants' remarks, requests for extension of time, and any other distinct papers be submitted on separate pages.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ganapathirama Raghu whose telephone number is 571-272-4533. The examiner can normally be reached between 8 am-4: 30 pm EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Nashaat T. Nashed can be reached on 571-272-0934. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300 for regular communications and for After Final communications. Any inquiry of a general nature or relating to the status of the application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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Patent Examiner
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